

## **AMENDMENTS TO THE SPECIFICATION**

**On page 1, line 1 of the Specification, please add:**

This application is a National Phase Application under § 371 of International Application Number PCT/EP03/07602 filed on July 14, 2003.

**Please amend the paragraph at top of page 2 as follows:**

A live, non-virulent strain of *Arthrobacter* (a member of the family of Corynebacteria) is marketed ~~under the name "Renogen"~~ in as a vaccine intended to protect salmon and other farmed fish against bacterial kidney disease (BKD). The characteristics of this strain are disclosed in WO 98/33884. This vaccine is unique in that it is the first live culture to have been licensed for use in aquaculture.

**Please amend the second and third paragraphs on page 8 as follows:**

In the Experiment described in Example 2, an abundant *Arthrobacter* surface protein of about 67kDa that is apparently linked to cell wall peptidoglycan was analysed by N-terminal amino acid sequencing. The N-terminal amino acid sequence of 20 residues was found to be identical to that of *Mycobacterium* hsp70, and this 67kDa protein is believed to correspond to the hsp70 protein of the present invention. In one aspect the invention provides an isolated heat shock protein of approximately 67kDa measured by SDS-PAGE which is localized to the cell wall of *Arthrobacter* cells and has the N-terminal amino acid sequence: (M)SRAVGIDLGTTNSVSVLE (SEQ ID NO: 3).

The results of our experiments demonstrate that the immunogenicity of *Arthrobacter* hsp70 in fish is comparable with that of *Mycobacterium* hsp70 in mammals. With the benefit of hindsight, we now believe that *Arthrobacter* hsp70 accounts to some degree for the disease protection conferred by ~~the Renogen™~~ a live *Arthrobacter* vaccine when used to vaccinate fish.

**Please amend the first and second paragraphs on page 20 as follows:**

Areas of greatest similarity between several mycobacterial and streptomyces hsp70 (dnaK) sequences at the nucleotide level are used to design degenerate primers for PCR and sequencing. The selected primers are dnaK-1Fdeg (5'-gtcggnatcgacctvggnac-3') (SEQ ID NO: 4) and dnaK-4Rdeg (5'-gcggtsggctcggtgac-3') (SEQ ID NO: 5). These primers are used for amplification of *Arthrobacter* DNA in a PCR reaction with a 50°C annealing temperature. The quality of the amplified DNA is assessed by gel electrophoresis. A 10µl aliquot is electrophoresed on a 0.8% agarose gel in 1X Tris-borate electrophoresis buffer (TBE) at 100V for about 1 hour. The 650bp product is then excised from the gel and purified using the Qiaquick purification kit (Qiagen). The PCR product is cleaned using Qiagen PCR clean up kit according to the manufacturer's instructions) and sequenced according to the manufacturer's instructions using BigDye primer chemistry (Applied Biosystems) and each of the primers used for the PCR. The extension reaction mixtures are prepared using the ABI PRISM (8r) BigDye Terminator Cycle Sequencing Ready Reaction mix, ~ 600ng of DNA template, 3.2 pmol of the appropriate primer and ddH<sub>2</sub>O to 20 µl. Conditions for cycle sequencing are as follows: the thermal cycler is set to 25 cycles consisting of 96°C for 10s, 50°C for 5 s, and 60°C for 4 min. The sequence shows this fragment to contain the first approx. 400bp of the dnaK gene.

In order to obtain additional hsp70/dnaK gene sequence, degenerate primers are used to amplify a downstream portion of the *Arthrobacter* hsp70 gene. These are selected from Galley et al. (1992) Biochemica et Biophysica Acta 1130: 203-208: [Forward primer hsp70 universal-F1 (5' CAR GCN CAN AAR GAY GCN GG 3') (SEQ ID NO: 6), Reverse primer hsp70 universal-R1: (5' GCN CAN GCY TCR TCN GGR TT 3') (SEQ ID NO: 7)]. The primers are designed to anneal to two highly conserved regions within the hsp70 gene to generate a 650bp product.